Research Note

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First documented pest outbreak of the herbivorous springtail *Sminthurus viridis* (Collembola) in Europe

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Running header: Outbreak of *Sminthurus viridis* in Europe

Abstract

Sminthurus viridis (Collembola: Sminthuridae) is a native of grasslands across Europe and feeds preferentially on clover (*Trifolium* spp.) and lucerne (*Medicago sativa*), although its abundance does not normally reach damaging pest levels (as occurs in Australasia). This research note describes the first quantitative assessment of a pest outbreak of this springtail in Europe, which occurred within an existing experiment investigating the effects of cultivation practices on forage establishment. Using sticky traps to assess the incidence of *S. viridis* we found a significant outbreak consisting of catches that were ten–fold greater than background levels in nearby, undamaged fields. Within the experimental area, lucerne established by direct drilling with herbicide had the highest incidence (105 (± 4.9) individuals per trap) compared to other treatments (79 (± 3.9)). Results are discussed in terms of how cultivation practice may have imbalanced the ecosystem; for example, herbicide use may have diminished potential refugia for predators during forage establishment. This paper highlights the potential of a currently innocuous, widely established invertebrate to become present at damaging levels in agricultural crops.

Keywords: agricultural pest, clover springtail, lucerne flea, Collembola, lucerne, direct drilling.

Introduction

The clover springtail, *Sminthurus viridis* L. (or lucerne flea), is an economically damaging invasive agricultural pest in Australasia (Bell and Willoughby, 2003), having been accidentally introduced from Europe in the 1880s (Hopkin, 1997). A pest outbreak is defined to have occurred when an insect population erupts to high densities, causing damage (Berryman, 1982). Herbivorous pest outbreaks often occur in non-native habitats; however, it is less likely for this to occur where they are native, because of the biological controls already in place (Berryman, 1982).

Sminthurus viridis feeds on clover (*Trifolium* spp.), lucerne (*Medicago sativa*) and other legumes and has a ubiquitous distribution across the Palaearctic, South Africa and North America (CAB International, 1973) but is not considered an agricultural pest in these regions. It has been found to occur at densities of up to 60 per 0.5 m^2 (through suction sampling) in agricultural fields in the UK (Alvarez *et al.,* 2000) and up to 70 per m^2 (in soil samples) in southern France (Renaud *et al.,* 2004). Historically, field damage by *S. viridis* alone has rarely been observed, quantified or monitored in Europe (Davies, 1928), with *S. viridis* referred to as an insect of 'minor economic importance' (Jones and Jones, 1984). However, some studies investigating the general effect of invertebrate herbivores, on clover have found damage caused by *S. viridis* (Mowat and Shakeel, 1989a, b; Wiech and Clements, 1992).

In Australasia, *S. viridis* can reduce stocking density by 0.6 sheep ha^{-1} through lost plant material (Wallace and Mahon, 1963) and is still causing more than $A28 million $annum^{-1}$ of damage to cereals and brassica crops there (Murray *et al.,* 2013). The abundance of *S. viridis* reported to cause economic damage is highly variable, ranging from 215-1300 per m^2 (Cleland, 1955; Bishop *et al.,* 2001) with some populations in untreated Tasmanian pastures reaching 50,000 per m^2 (Ireson, 1984). While this research note represents only one field site, in one location, it is the first quantitative assessment of a pest outbreak of the springtail *S. viridis* in Europe. The outbreak was captured within an experiment investigating different cultivation techniques, showing the wider implications of the phenomenon of pest outbreaks in native, widespread and common invertebrates in agricultural land, and suggesting a need for greater awareness and surveillance of new outbreaks globally.

Materials and methods

In June 2013, an experiment was set up in a 2 x 2 factorial structure, with a randomized complete block design, to evaluate the effect of different cultivation techniques (ploughing versus direct drill; with/without a pre-cultivation herbicide) on the yield of lucerne (n = 3). Each replicated plot was 10 x 2.7m, 0.5 m apart within block and each block was 7 m apart. The experimental field site was located near Aberystwyth, Mid–Wales, UK (52°26'2N, 4°1'W; elevation 30 m a.s.l.) on a silty clay loam. Previously the area had supported a perennial ryegrass sward, sown in 2007. Plots were randomly allocated for herbicide application and were treated on 10 June 2013 with 360 g L^{-1} glyphosate (Clinic Ace, Nufarm UK Ltd, Belvedere, Kent, UK) at a rate of 4 L ha^{-1}. Plots allocated for ploughing were ploughed to a depth of 150 mm, power–harrowed and rolled on 18 June, prior to surface sowing using a Fiona D784 seed drill (Westmac Maskinfabrik A/S, Bogense, Denmark) then lightly harrowed and rolled using a flat roller. Plots that had been randomly allocated for direct drill were sown with a Duncan

Eco Seeder direct drill (Willow Farm Machinery Ltd., Ludford, UK) into slots 15 mm deep. All plots were sown with lucerne (cv. Timbale) at a rate of 22 kg ha^{-1} on 19 June.

On 3 July 2013 an increased abundance of a jumping invertebrate was observed on the plots and damage to leaves was visible. This damage was characteristic of *S. viridis* with the epidermis stripped off the leaf and underlying mesophyll eaten, skeletonizing the leaves (Barker, 2006) (Figure 1). Two groups of specimens were randomly collected using an aspirator (Pocket Pooter, Watkins and Doncaster, Leominster, UK), from the 'outbreak' and identified. One group were identified using morphology (Hopkin, 2007) and confirmed by the UK recorder of Collembola as *S. viridis*. A second group were taken for molecular identification, by comparing individual cytochrome c oxidase subunit I (COI) sequences to known, publically available sequences for *S. viridis* (following the methods of Shaw and Benefer (2015)) to assess similarity to known 'pest' *S. viridis* from Australasia and determine whether they may be a separate pest morph – potentially explaining why this species does not usually reach pest proportions in Europe.

The unusually high abundance observed led to an assessment of numbers to ascertain whether this was comparable to any other reported outbreak. To assess abundance, a modified method previously used for assessment of hopping insects (Norment, 1997; Matsukura *et al.,* 2011) and which did not require destructive sampling of experimental plots, was set up on 4 July 2013. Yellow sticky boards (Silvandersson, Sweden) were cut in half (12.5 cm x 5.5 cm) and the halves placed centrally within each plot, ~2m apart (n = 24). The boards were horizontal, with one side adhering to the soil surface and staked centrally, whilst the other sticky side could catch jumping

Collembola, akin to pitfall trapping. These sticky boards were left in the field overnight (~15h) before removal. On 5 July, cypermethrin insecticide was applied at a rate of 200 ml ha^{-1} to salvage the original experiment (results of original experiment reported in Marley et al., 2015). Invertebrates attached to the sticky boards were identified and counted under a microscope.

To establish whether the outbreak could be considered of pest proportions, an area of established lucerne (sown 2011) of the same cultivar (approx. 100 m from the experimental plots) was used to compare populations (this site had no reported pest problems in the previous two growing seasons). Here, the same sticky board method was implemented (n = 12), over the same time scale and area. In addition, soil cores (5.7 cm diameter; n = 3) were taken from the outbreak, in the replicated herbicide treated (plough/direct drill) plots, prior to the application of the insecticide, to obtain an estimate of abundance per m^2 for comparison to previous estimates of damaging population levels. These were placed upside down on Tullgren funnels following Crotty *et al.* (2014) and the results transformed to per m^2 (by multiplication of 97.97) for comparison with other published results.

All data were analysed using GenStat (v14, VSN International, Hemel Hempstead, UK), *S. viridis* counts per trap were normally distributed (tested within GenStat) and assessment of the effect of treatment on catches was performed by a general analysis of variance (ANOVA) as a 2 x 2 factorial in a randomized complete block design, multiple comparisons were made using the Student Newman Keuls test.

Results

There was a ten–fold difference in the number of *S. viridis* caught in the newly planted lucerne crop (mean 86.8 (± 3.53) n=24) compared with a 'normal' population (mean 6.2 (± 1.28) n=12) in an established lucerne crop ($P < 0.001$). There were also significant differences among management treatments ($P = 0.005$). More *S. viridis* were caught in the direct drill-with-herbicide plots (mean average 105.5 (± 4.92)), compared to the other treatments (DD without herbicide 74.5 (± 5.22), plough without herbicide 82.3 (± 10.19), plough with herbicide 81.3 (± 4.19)). No significant difference was found when comparing the cultivation method alone (plough 81.8 (± 5.26) vs direct drill 90.0 (± 5.79)) ($P = 0.072$). However, herbicide usage was significant when considered singularly ($P = 0.007$; mean catches in herbicide plots were 93.4 (± 4.77) compared to 78.4 (± 5.58) in plots without herbicide). There was a mean of 261 (± 130.6) *S. viridis* per m^2 extracted from the soil cores.

COI sequences were obtained for seven individuals, ranging from 593–681bp in length (GenBank accession numbers KJ155509–KJ155515). They produced a 92.6–98.7% match to *S. viridis* sequences on BOLD (Barcode of Life Database), all to individuals collected from New Zealand, (Figure 2). At this time there are no other UK sequences for *S. viridis* on BOLD/GenBank.

Discussion

Our study shows *S. viridis* at pest levels in its native environment, with results showing ten–fold greater catches in the affected area than a 'normal' population and damage being caused (Figure 1). We have measured this infestation within an experiment with replicated treatments showing the potential effects of agricultural management on pest abundance. Prior to the outbreak, the weather conditions over the previous 12 months had shown large fluctuations from the long-term average. Data provided by the Met Office, for the weather station located at Gogerddan Aberystwyth, showed that 2012 was wetter than the 50-year average, whilst spring 2013 was drier and colder than the 50-year average. These extremes were experienced across the UK (Met Office 2013a; 2013b), and may have been a contributing factor to this pest outbreak. This study is limited by being representative of only one field site, during the first establishment year of a *S. viridis* host plant; however, we are aware of at least one other occurrence in a different location during the same growing season (R. Fychan, personal communication). Our findings highlight the need for greater awareness and surveillance of potential pest outbreaks, especially where these may be exacerbated by changing weather conditions as projected for the UK by current models (IPCC, 2014).

Comparisons between an established crop ('normal' population) and the affected experimental plots confirmed that there was an outbreak of *S. viridis* with an order of magnitude difference between catches (mean average of 6.2 (± 1.28) in a 'normal' population, compared to 86.8 (± 3.53) in the outbreak). It also verified that sticky boards could be used to test the incidence of *S. viridis* in the field. This method was previously found to be comparable to pitfall trapping of surface-active arthropods (Norment, 1987); is already used for other hopping pests of crops (e.g. plant and leaf hoppers (Matsukura *et al.,* 2011)), and is easier to use than a sticky trap corer (Taverner *et al.,* 1996). Sticky traps set in the direct-drill-with-herbicide treatment caught the greatest numbers overall; possibly due to the reduced plant cover diminishing refugia for surface dwelling predators, whilst also stimulating crop growth through reduced competition (Wardle, 1995). In comparison, the direct drill without herbicide treatment had the most 'intact' ecosystem, with the lucerne competing with existing ryegrass and potentially allowing greater numbers of predators to reside within these plots, thereby reducing *S. viridis* abundance.

The abundance of *S. viridis* reported to cause economic damage is highly variable, although can be as little as 215 per m^2 (Cleland, 1955). If this is true, without the immediate application of insecticide substantial yield reductions would have occurred here (results from soil cores estimated 261 (± 130.6) individuals m^2). The few individuals of *S. viridis* obtained via soil cores demonstrates that this is not the most appropriate method for ascertaining abundance of an epigeic springtail because of its ability to jump away from the soil core during sampling. Here, the sticky board method has been shown to be a useful 'farmer friendly' tool that could be implemented in the field to quickly assess population size of *S. viridis*. However, the methods of Alvarez *et al.* (2000) or use of other suction-based methods may provide a more accurate assessment as they provide a per-m^2 measure of abundance.

The COI sequences obtained were a high match to other COI sequences available on BOLD and were in agreement with the morphological identifications, confirming the identity of the species causing the infestation. All individuals matched either haplotype 1B (98.6–98.7% match) or haplotype 2B (92.6% match) from New Zealand (Vink and Brown, 2013; Figure 2). Due to the paucity of UK and European sequences published for *S. viridis*, we could not ascertain whether a ‘pest morph’ was present, as opposed to a ‘normal’ ubiquitous European morph.

In European grasslands, persistence of forage legumes can be unreliable. Historic studies and surveys have found 90% of clover leaves with pest damage at some sites, and up to 30% of this damage was due to unidentified pests (Lewis and Thomas, 1991). The addition of a pesticide was also shown to increase dry matter yields of white clover, although not of lucerne, however the pests were not identified (Clements and Henderson, 1983). It is possible that this unreliability is because of undiagnosed *S. viridis* outbreaks occurring, as suggested by Mowat and Shakeel (1989b), but due to lack of research and publicity they remain undetected. Overall, it was found that crop management can alter the abundance of a ubiquitous, usually harmless springtail, to pest proportions. These findings highlight the need for further understanding of the different agricultural and environmental interactions affecting *S. viridis* populations globally. This outbreak could be a rare localized occurrence or the harbinger of future pest outbreaks, and only greater awareness and surveillance will help to understand this issue within agricultural ecosystems globally.

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FIGURE LEGENDS:

Figure 1: Photograph of damage to lucerne leaves caused by *S. viridis.*

Figure 2: Phylogenetic tree using maximum likelihood in MEGA v5 (Tamura *et al.,* 2011) of cytochrome c oxidase I subunit I (COI) sequences of *Sminthurus viridis* using the TN92+G model (LogL = -2019.18) using the obtained sequences and those for *S. viridis* downloaded from the Barcode Of Life Database (BOLD) and GenBank to determine intraspecific relationships between samples collected in Europe and Australasia. The percentage of trees in which the associated taxa clustered together is shown next to the branches, and branch lengths are representative of the number of substitutions per site. There were 428 positions in the final dataset. C169-C175 represent *S. viridis* individuals collected from the infested field site in Aberystwyth (accession numbers KJ155509 – KJ155515). All other sequences were downloaded from BOLD (accession numbers K150049 – KC150080) and GenBank (accession numbers EU016192, JN970939 and HM355586 – HM355589). *Sminthurinus trinotatus, S. aureus and S. elegans* were included as outgroups (accession numbers pending; Shaw and Benefer, 2015).